Effect of Desoxycorticosterone upon the Toxic Actions of Somatotrophic Hormone. (18539)

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Earlier animal experiments had shown that lyophilized anterior pituitary (LAP) material causes nephrosclerosis, polyuria as well as lesions in the cardiovascular and connective tissues which resemble those seen in the so-called "collagen diseases" of man(1,2). In these and many other respects, LAP imitated the actions of mineralo-corticoids, such as desoxycorticosterone acetate (DCA)(8) or desoxocortisone(3). Since LAP is very active in causing adrenocortical enlargement, it would have been tempting to ascribe its above mentioned "DCA-like" effects to its corticotrophin content. However, later, when adequate amounts of pure ACTH became available, it was noted that even the highest tolerable doses of the latter failed to reproduce these actions of LAP. Therefore we were forced to conclude that the mineralo-corticoid-like properties of LAP must be due to some other factor present in the pituitary preparation, which was merely referred to as the "LAP-factor" or "X-factor" of the anterior lobe(4). Since LAP is especially rich in the so-called "growth hormone" or somatotrophic hormone (STH), we compared the actions of our LAP with those of electrophoretically pure STH (kindly supplied by Professor C. H. Li of Berkeley, California). A large series of pertinent experiments showed that the latter reproduces, with great fidelity, all the DCA-like actions of the crude LAP preparations(5-7). The question arose whether STH elicits these effects by inducing the adrenal cortex to produce mineralo-corticoids—that is through a special type of corticotrophic action —or whether there is a synergism between independently produced mineralo-corticoids and STH, in the peripheral receptor cells themselves.

In the present communication we wish to report upon experiments in which STH and DCA were administered, both singly and in combination, in order to examine a possible synergism between them.

Materials and methods. Twenty-five female piebald rats weighing 85-110 g (average 97 g) were used as *experimental animals*. In all of them the right kidney was removed on the day preceding the administration of the first injections. The animals received a powdered "Purina Fox Chow" diet and were given 1% NaCl solution instead of drinking water. The unilateral nephrectomy and the NaCl supplement were indicated by earlier experiments which showed that these factors increase sensitivity to the nephrosclerotic action of LAP(4). On the day following the unilateral nephrectomy the rats were subdivided into four groups as follows: I normal controls, II received STH alone, III were given DCA alone and IV STH and DCA simultaneously but injected at different sites.

The *STH preparation* used was one supplied by N. V. Organon (Oss) bearing the lot number CH I Kr. and was prepared by Professor Dingemanse of Amsterdam (Holland). It had been sent to Dr. Pedersen of Upsala (Sweden) who examined it by the ultracentrifuge method and found it to consist of two fractions, one representing 67% and other 33% of the material. This, as well as other tests performed by Organon (Oss), led the firm to conclude that the preparation consists of 67% STH and 33% of contaminating material. This preparation was dissolved in 1% NaCl, in such a manner that each individual dose was contained in 0.2 cc of the solvent. 5 mg per day, divided into 3 subcutaneous injections, were given once every 8 hours

1. Selye, H., *Canad. M.A.J.*, 1944, v50, 426.
2. Selye, H., *J. Clin. Endocrinol.*, 1946, v6, 117.
3. Selye, H., *Brit. M. J.*, 1950, v1, 203.
4. Selye, H., 1950, "STRESS" Acta Inc. Med. Publ., Montreal.
8. Selye, H., and Pentz, I., *Canad. M.A.J.*, 1943, v49, 264.

TABLE I. Summary of Organ Weights.

Groups		I Normal controls	II STH	III DCA	IV STH+DCA
No. of rats per group		6	6	6	7
Initial body wt (g)		97	97	97	99
Final body wt (g)		126 ± 6.6	169 ± 6.4	111 ± 7.3	122 ± 10.2
Heart	mg	486 ± 35	664 ± 34	506 ± 25	620 ± 48
	mg/100 g	389 ± 26	392 ± 9.8	463 ± 33	511 ± 14
Kidney	mg	747 ± 54	1179 ± 113	940 ± 36	1447 ± 162
	mg/100 g	591 ± 14	693 ± 49	859 ± 51	1184 ± 96
Liver	mg	6313 ± 564	10681 ± 481	5953 ± 194	7720 ± 739
	mg/100 g	4975 ± 201	6331 ± 207	5450 ± 351	6322 ± 246
Adrenals	mg	30 ± 2.2	55 ± 2.1	24 ± 1.3	51 ± 4.2
	mg/100 g	23 ± 0.09	32 ± 1.1	22 ± 2.7	42 ± 2.3
Thymus	mg	208 ± 21	270 ± 13	134 ± 21	37 ± 9
	mg/100 g	164 ± 10	161 ± 11	121 ± 17	28 ± 4.8
Spleen	mg	1111 ± 54	2031 ± 188	965 ± 153	723 ± 140
	mg/100 g	893 ± 57	1211 ± 116	840 ± 100	566 ± 73

throughout the day and night, in order to obtain a relatively constant overdosage.

Desoxycorticosterone acetate was administered in the form of microcrystals as prepared by Merck and Co. (25 mg per cc), suspended in saline solution with added suspending agents. Of this 5 mg per day was injected subcutaneously once daily. Only the water intake was measured daily, as an index of *water turnover,* since the accurate collection of urine is technically difficult in the rat. In Table II the corresponding figures represent the mean values of the last seven days of observation.

Experimental results. The experiment had to be terminated after 14 days of treatment because all the animals receiving both STH and DCA (group IV) became extremely edematous and so weak that they obviously could not have survived much longer.

Changes in body and organ weights. In order to facilitate the evaluation of the changes observed, the weights of all organs have been expressed both in mg and in mg per 100 g of body weight with the standard errors.

In agreement with expectations, STH alone increased *somatic growth* very considerably. This weight increment was greatly counteracted by simultaneous administration of DCA, despite the manifest edema in group IV, which must have added considerably to the apparent weight of the rats. On the other hand, the enlargement of the *heart* and *kidneys* caused by STH was greatly augmented by simultaneous DCA treatment. The *liver* weight increase caused by STH was not further augmented by DCA. The "compensatory atrophy" of the *adrenals* caused by DCA was completely abolished by simultaneous STH treatment. Indeed, our STH proved to be strongly corticotrophic as judged by the adrenal weight increase, which it elicited when given either alone or in combination with DCA. Since this STH preparation was impure, its corticotrophic effect could have been due to contaminating ACTH; however, in our earlier work with electrophoretically pure STH this adrenal enlargement was also always very evident(5-7).

The *thymus* and *spleen* stimulating action of STH was actually diminished by simultaneous DCA administration, but this may be a non-specific effect due to the debilitated condition of the animals, with consequent endogenous ACTH liberation.

Histologic and clinical observations. The most important histologic and clinical observations are summarized in Table II.

Since in this experiment the animals were treated only during 2 weeks it is not unex-

5. Selye, H., *Brit. M. J.,* 1951, v1, 263.
6. Selye, H., *Am. J. Med.,* 1951, in press.
7. Selye, H., 2nd Clinical ACTH Conference, 1951, in press.

TABLE II. Summary of Some Histologic and Clinical Observations.

Groups	I Normal controls	II STH	III DCA	IV STH+DCA
Myocarditis	0	*	++	+++
Nephrosclerosis	0	+	+	+++
Pancreatic periarteritis	0	*	+	++
Water intake, cc	45 ± 1.6	93 ± 6.2	81 ± 3.9	163 ± 7.3
Subcut. and retroperitoneal edema	0	0	+	+++
Blood pressure	128 ± 3.0	148 ± 13	151 ± 12	129 ± 14

* Trace.

pected that neither STH nor DCA produced maximal histologic changes. Both these compounds, given singly, elicited the usual type of hyalinizing granulomatous myocarditis with periarteritis nodosa of the cardiac vessels, nephrosclerosis, inflammatory changes in the stroma and fat of the renal pelvis, proteinuria, and some extra-cardiac periarteritis nodosa, for instance in the pancreatic vessels, which are especially predisposed to this type of change. The water intake and diuresis was raised both by STH and by DCA alone, but only the latter produced a very mild degree of subcutaneous and retroperitoneal edema; even this was found only in some of the animals. On the other hand, conjoint treatment with STH and DCA resulted in a considerable potentiation of all these actions with marked myocarditis, nephrosclerosis and nephritis, pancreatic periarteritis nodosa, polyuria and edema formation. This marked sensitization of the cardiovascular and connective tissues by STH to the toxic effects of DCA may well account for the moribund condition of the animals in group IV. Both electrophoretically pure STH(5) and DCA(4) cause a rise in blood-pressure. This is confirmed by the present observations. The fact that conjoint treatment with the two hormone preparations did not result in any hypertension is pre-

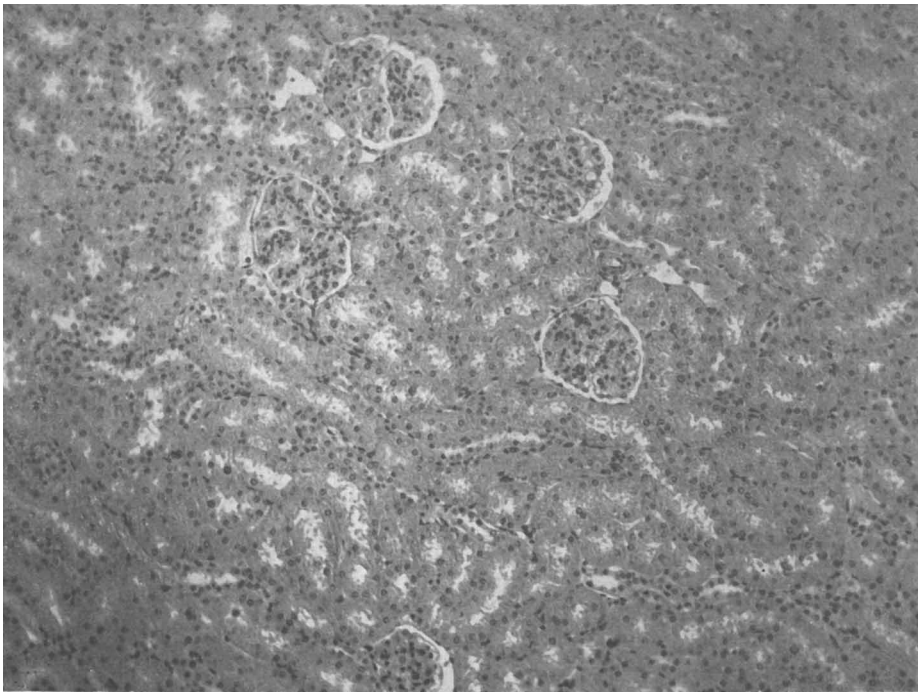

FIG. 1.
Kidney of normal control rat.

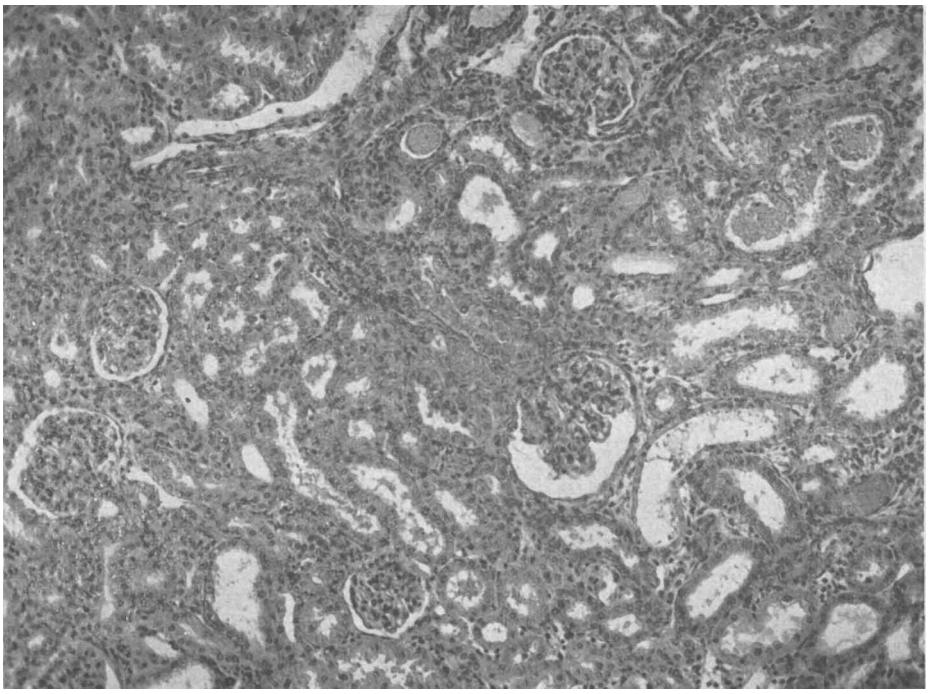

FIG. 2.

Kidney of STH-treated rat. Note dilatation of many tubules some of which contain hyalin casts or protein precipitates. The afferent arteriole of the central large glomerulus is almost completely hyalinized.

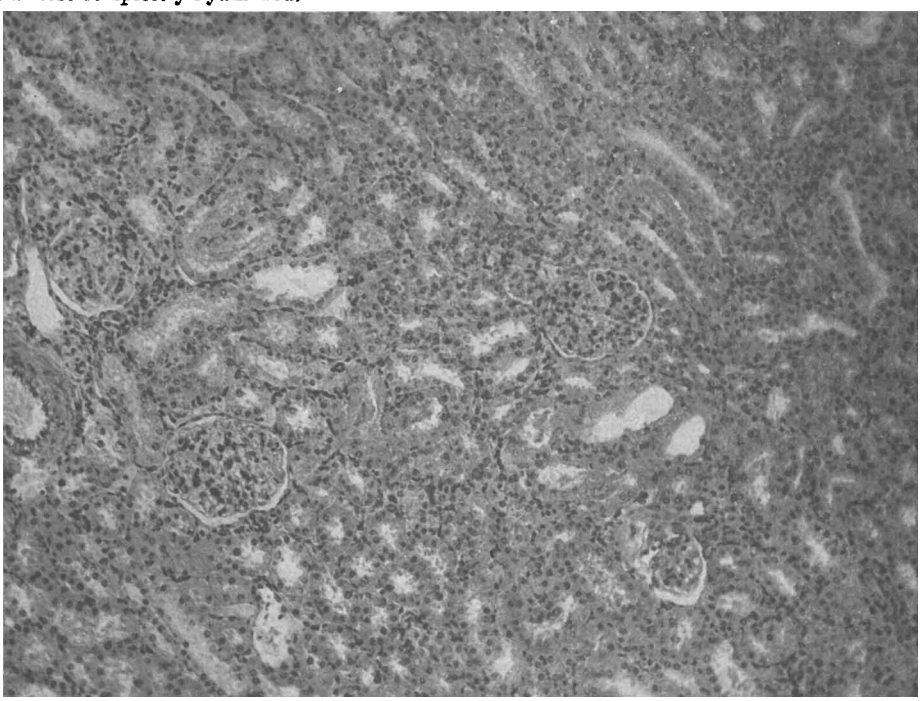

FIG. 3.

Kidney of DCA-treated rat. Note some tubular dilatation and protein precipitation, especially in the tubules situated in the center of the field. Surrounding this central region are several collapsed tubules exhibiting the appearance of "endocrine nephrons."

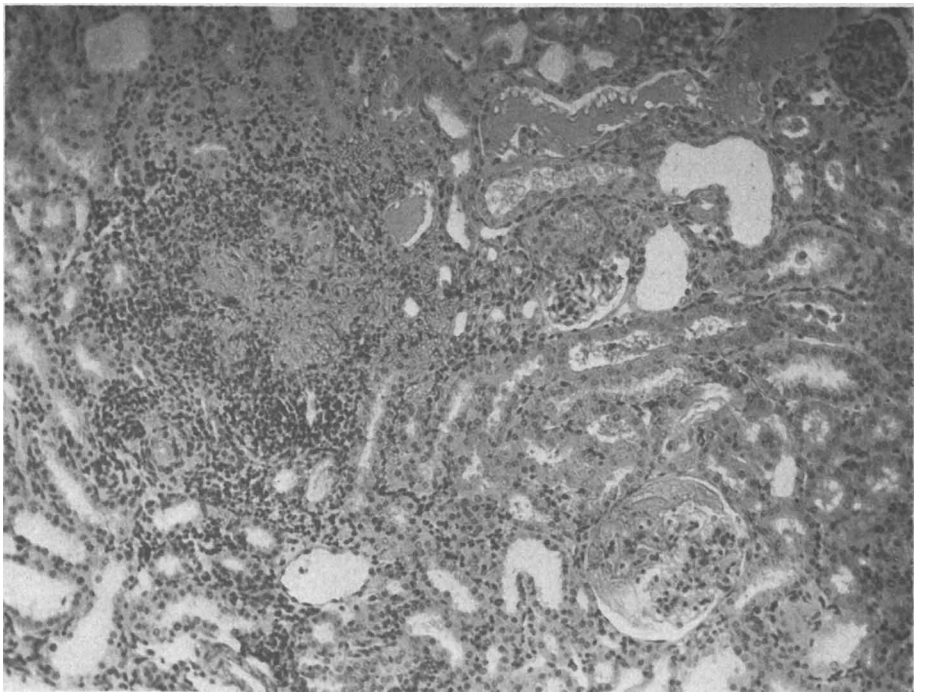

FIG. 4.
Kidney of rat treated with STH and DCA. Note one intensely hyalinized glomerulus in lower right corner of the field as well as several hyalinized blood vessels surrounded by an inflammatory granuloma in the upper left portion of the field. Most of the tubules are greatly dilated; some of them contain papillomatous excrescences, others proteinaceous material or hyalin casts.

sumably due to the poor physical condition of the animals.

Summary and conclusions. 1. Experiments on rats—sensitized to mineralo-corticoid actions by unilateral nephrectomy and a high sodium intake—showed that somatotrophic hormone (STH) shares with desoxycorticosterone acetate (DCA) the ability to produce: cardiac hypertrophy with histologic signs of a rheumatic-like myocarditis, hypertension, renal enlargement with microscopic evidence of malignant nephrosclerosis and an increased water turnover with proteinuria. 2. All these actions are greatly increased when STH and DCA are administered simultaneously, except for the rise in blood-pressure. The failure to develop hypertension under the influence of the two preparations may well be due to the fact that this hormone combination is extremely toxic and causes pronounced edema with obvious deterioration in the physical state of the animals. 3. It is concluded that STH sensitizes the tissues to the production of "collagen-disease-like" lesions by DCA. 4. STH also causes adrenocortical enlargement and prevents the compensatory atrophy of the adrenal cortex normally elicited by DCA. This effect may be due: to a contamination of our preparation with ACTH, to the compensatory secretion of ACTH by the pituitary of the STH-treated animal, or to a true corticotrophic action of STH. 5. Although earlier experiments had shown that even electrophoretically pure STH causes similar adrenal stimulation, we have no evidence either for or against the view that STH induces the adrenal cortex to produce mineralo-corticoids. The "mineralo-corticoid-like" actions of STH could result solely from the sensitization of the peripheral tissues by STH. Increased mineralo-corticoid production by the adrenal cortex under the influence of STH may be an additional factor, but its possible participation remains to be demonstrated.

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Electron Microscope Observations on Elastic Fibers. (18540)

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Elastic tissue generally considered both from chemical and submicroscopic analysis (1) as a relatively homogeneous entity, has been recently described as a two component system(2). Gross(2) in a very documented electron microscope analysis of elastic tissue from a variety of sources concluded that the elastic fibers are "composed of bundles of trypsin resistant threads of characteristic form and size plus a trypsin sensitive, heat-resistant 'amorphous' binding matrix." This conclusion was based on experiments in which digestion of either boiled or unheated elastic tissue with crystalline trypsin caused the "release" of characteristic thin coiled threads. These threads were observed in the surrounding of the partially digested fibers, but could not be observed with certainty within the fiber itself. In some recent experiments of digestion with crystalline trypsin on nerve material we were surprised to find threads which looked very similar to those described by Gross on elastic tissue. Furthermore, in control experiments in which the enzyme alone was incubated, similar threads could be observed, simultaneously with the presence of few bacteria and bacterial flagella.

These findings led us to reinvestigate the structure of the elastic tissue with the electron microscope, and to the demonstration that the threads described by Gross(2) are not elastic constituents.

Material and methods. The elastic material was obtained from the swimming bladder of the fish micropogon opercularis (Quoy and Gaimard). The preparation was similar to that described by Gross with minor modifications. The swimming bladder was cut into small pieces and stored overnight in 1% acetic acid at 4°C. The viscous mass obtained was diluted with more acetic acid and fragmented in a Waring Blendor. The elastin was separated from the ichthyocol (collagen) by repeated centrifugations and washing with dilute acetic acid. To complete the collagen extraction the suspension was boiled for 30 minutes and washed once more in acetic acid. The purified elastin was finally suspended in distilled water and boiled once more for sterilization. For this last operation glass beads had to be added to the suspension to overcome the strong tendency to stick of the purified elastin. Crystalline trypsin (Armour) dissolved to 0.1% in borate or phosphate buffer of pH 8 was used. Some digestion experiments were made under toluene, as described by Gross. In other cases a crystal of thymol was added to the fresh solution.

Digestion experiments under complete aseptic conditions were also performed. The trypsin solution was filtered through a Seitz microfilter. The buffer solution was sterilized by boiling and a final check of the pH value was made. Sterile glass material was used and all the operations were performed aseptically. The tubes containing the elastic material together with the controls were incubated at 37°-38°C for the same amount of time. After digestion the suspension was slightly disintegrated with a 10 Kc sonic magnetostriction oscillator. Specimens for electronmicroscopy

1. Wolpers, C., *Klin. Woch.*, 1944, v23, 169.
2. Gross, J., *J. Exp. Med.*, 1949, v89, 699.